NITROGEN IN WOOD AND ITS ROLE IN WOOD DETERIORATION[1]

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Abstract

Based on present knowledge of the origin, amounts, chemical form, and distribution of nitrogen (N) in wood, hypotheses are proposed to explain radial gradients in N content that exist across the xylem cylinder of tree stems: (1) N in the cytoplasm of developing wood cells is diluted by apposition of cell wall substances; (2) after maturation of wood fiber cells, N in their cytoplasm is removed by elution into the transpiration stream; (3) death of xylem parenchyma cells during aging of sapwood and formation of heartwood is accompanied by removal of much of the N in their cytoplasm. Hypotheses 2 and 3 above suggest strongly that trees possess an internal recycling mechanism for conservation and reuse of the N in the cytoplasm of xylary cells.

Although the supply of N in wood is meager, wood-destroying fungi readily metabolize the carbon-rich constituents of wood and produce large fruiting structures that release vast numbers of spores in nature. To account for these capacities, we postulate that these fungi employ one or more of the following three mechanisms: (1) preferential allocation of N obtainable from wood to substances and pathways highly efficient in the use of wood constituents; (2) reuse of N obtainable from wood by a dynamic and continuous process of autolysis and reuse without significant loss of N; (3) utilization of N sources outside the wood itself, for example, by fixation of atmospheric N.

Introduction

One of the most intriguing aspects of the disintegration of plant tissues by microorganisms is the capacity of wood-destroying fungi to metabolize the carbohydrates, lignin, and other carbon-rich constituents in wood which almost invariably contains very meager amounts of nitrogen (N). In contrast with herbaceous tissues that typically contain 1–5% N by weight, woody plant tissues usually contain only 0.03–0.10% N (4, 21). The C:N ratio of most wood species is (350–500):1 and in some species is even higher, 1250:1, in the highly decay-susceptible heartwood of sitka spruce, for example (34). For most saprophytic microorganisms, a medium with so high a ratio of C:N would be deficient in N and would support very little growth; but wood-destroying fungi apparently are well adapted to wood as a substrate despite its meager N content.

Nitrogen is just as essential for the growth and development of wood-destroying fungi as it is for all other living organisms. In fungal hyphae and spores, N is a major constituent of the amino acids, peptides, and proteins (about 16% N), purine and pyrimidine bases (25–52% N) of the nucleic acids, chitin (6.9% N), and other compounds. Since N constitutes a substantial fraction of the dry weight of active fungus tissue (16), wood-destroying fungi apparently must utilize large amounts of wood substance to obtain sufficient N for production of the abundant vegetative mycelium, large fruiting structures, and enormous numbers of spores observed in nature.

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[1] This paper is the first in a series on the role of nitrogen in wood deterioration. The second and third papers have been submitted to Phytopathology and to this journal, respectively.

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Wood-destroying fungi presumably satisfy their N requirements primarily from the wood itself. Pure cultures will decay sterile wood blocks with no exterior sources of N except the atmosphere (87); but none has been shown to fix atmospheric N. Quantitative analyses show that the amount of N in the deteriorating residue of wood substance plus contained fungus mycelium does not change as decay progresses and is essentially identical with that of undecayed wood (42, 50). Thus, wood-destroying fungi apparently have developed very efficient mechanisms for the assimilation, utilization, and conservation of N in wood during decay.

This paper has been prepared to provide a foundation for elucidation of these mechanisms. It contains a synthesis of existing knowledge concerning the origin, amount, distribution, and chemical form of N in wood, the N content of fungus mycelium and spores, the metabolism of N by wood-destroying fungi, and the influence of N on the decomposition of woody plant tissues. Based on this information, a series of working hypotheses are proposed as a basis for much-needed fundamental investigations of these important aspects of saprogenesis.

Knowledge of the role of N in deterioration of woody plant tissues has importance in the ecology and physiology of wood-inhabiting fungi, the biochemistry of plant tissue disintegration, and, since plant tissues without N theoretically would be immune to decay, possibly also in determining the natural resistance of wood to microbial deterioration and in the development of new methods to protect wood from decay.

Nitrogen in Wood

Nitrogen in wood is mentioned only in passing in the major published literature of wood chemistry (13, 88) and tree physiology (54, 84, 89). Studies in cambial chemistry have provided some information on the origin of N in wood (33). Several authors have determined the amount of N in mature woody tissues (4, 10, 74, 82), but most such studies have ignored the relevant contributions of cambial chemists as well as the gradients in distribution of N in tree stems that have been demonstrated or can be deduced from knowledge of wood anatomy. Except for the alkaloids in a few tree species (65), very little is known of the chemical structure of the nitrogenous constituents of woody plant tissues. This lack of knowledge is an understandable consequence of the very small amounts of N present in wood and the negligible importance of N in technical aspects of wood utilization. But N is absolutely essential both for the synthesis of woody tissues and their degradation by microorganisms and insects. Thus, stimulus for investigation of N in wood apparently will derive mainly from its importance in these areas (32).

Nitrogen in wood usually is attributed to proteins considered to be part of dried remnants of the cytoplasm in dead wood cells (13, 88). The inadequacy of this generalization is apparent immediately from the mechanisms of synthesis of woody plant tissues and knowledge of tree physiology.

Origin of Nitrogen in Wood

Nitrogen in wood originates in the cytoplasm of the cambial cells that differentiate and mature into woody plant tissues. Changes in N content that take place during various stages in the maturation of these cells are shown diagrammatically in Fig. 1. The major points in this double-logarithmic plot for a hypothetical tree, 50 years of age and containing 10 annual increments of sapwood, are consistent with experimentally determined values for N in conifers (9, 33, 69). The major phases of diminution in N content have been named according to the predominant mechanisms that we believe to be involved at various stages after cell division. Actual N-content values for a given wood species or individual tree may differ considerably from those shown in Fig. 1, but the general trends described here apply equally well to temperate zone angiosperms and gymnosperms. Factors that we believe to be responsible for changes in N content associated with each phase are described below.

Cells in the cambial zone contain cytoplasm rich in all the enzymes and other proteins, peptides, amino acids, nucleic acids, lipoprotein membranes, and other nitrogenous constituents characteristic of meristematic cells in higher plants. In addition, certain proteins that function in cell wall extension are believed to be deposited within the primary walls of differentiating cambial cells (58). In common with living cells in higher plants in general, the N content of the cambial zone varies with the individual tree, its species, nutritional status, and other factors, from 1 to 5% by weight (21).

After the cambial initials extend in length, they develop secondary walls and become lignified. During these phases of development, the finite amount of N in the cytoplasm and primary walls of these highly vacuolated cells may not change appreciably, but its percentage by weight decreases markedly as the protoplasm is diluted by progressive apposition of cellulose, lignin, and other secondary cell wall substances (82). In Fig. 1 we have used the term "dilution phase" to refer to the diminution in N content during the stages of cell enlargement and cell wall formation in the maturation of wood cells. The dilution phase continues until the process of lignification is complete, usually about 10 to 30 days after cell division at the cambium. Dilution can be visualized readily by examining cross sections of differentiating cells in any tree species (48). The magnitude of dilution has been measured by comparing the N content of tissues identified as xylem scrappings and stringy xylem beneath the cambium and found to be approximately 10-fold in two gymnosperms and three- to six-fold in two angiosperms (33).

After lignification, vascular elements (wood fiber cells) that comprise the bulk of the xylem cylinder in temperate-zone species quickly die and apparently lose their cytoplasm with its N-rich constituents and organelles. The rapidity of this loss (at least in certain cases) is illustrated well in the electron micrograph in Fig. 2, which shows two adjacent cells about 10 cells in from the cambium, just beyond the zone of lignification in a developing stem of *Pinus radiata* D. Don. One cell contains cytoplasm with apparently intact vacuolar and plasma membranes and other cytoplasmic organelles, while the other cell shows no evidence of cytoplasmic constituents of any kind. The fate of the cytoplasm in maturing vascular elements has not been demonstrated specifically (18), but lack of cytoplasm is typical of the appearance of mature wood cells in both the light microscope and the electron microscope (18, 20, 53, 59, 60, 62, 77, 85). This argues strongly against the conclusion that the bulk of the N in wood usually exists as a dried remnant of the cytoplasm, at least in wood fiber cells; it appears much more plausible to postulate that the cytoplasm disappears from the vascular elements.

It has been suggested (61, 85, 86) that the so-called warty layer found in certain coniferous species may be formed by apposition and adherence to the cell wall of cell organelles enclosed between the plasma and vacuolar membranes. In many cases, however (as in Fig. 2), the warts are part of the cell wall itself and distinct from the membranes of the cytoplasm (18, 19, 61).

The mechanism by which we believe the membranes and organelles of the cytoplasm lose their integrity and are removed from the wood fibers will need to be demonstrated experimentally. It is logical to postulate, however, that an active process of autolytic depolymerization is the mechanism of disintegration and that it results in solubilization of the cytoplasmic constituents. As the cells become functional in conduction, these solubilized

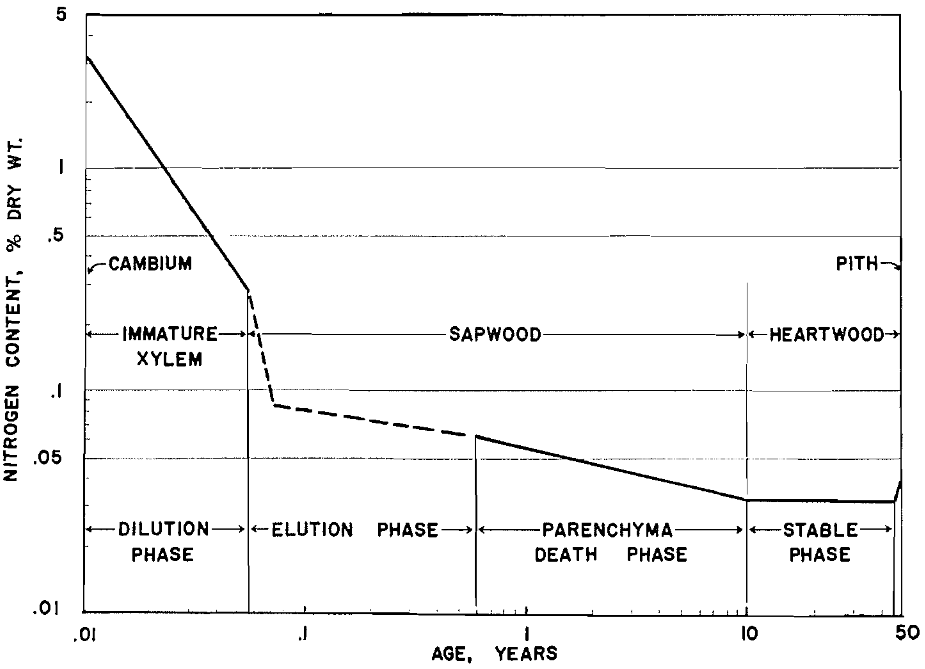
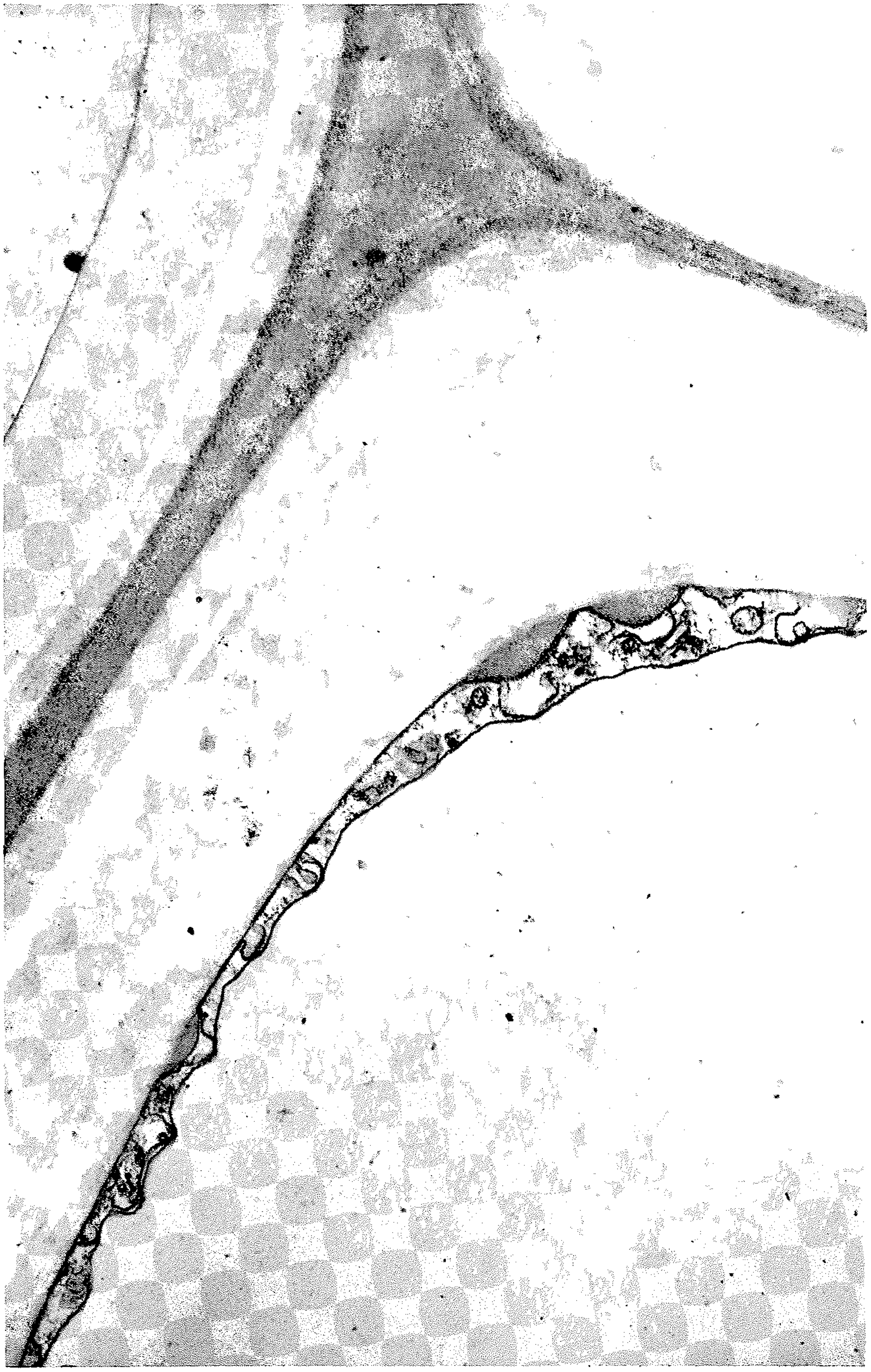


FIG. 1. Hypothetical graph of changes in nitrogen content during various stages in the maturation and aging of the xylary tissues in a typical angiosperm or gymnosperm. The major points on this hypothetical curve are consistent with experimentally determined values for N in conifers. The major phases of change in N content have been named according to the predominant mechanism we believe to be involved at various stages after cell division in the maturation of wood cells.

FIG. 2. Cross section of developing tracheids in a file of cells just beyond the zone of lignification in a stem of *Pinus radiata* D. Don. Note intact cytoplasm in one cell and lack of cytoplasm in the other cell.



constituents then would be eluted in the transpiration stream and carried upward. Considering the huge volume of tracheal fluid that traverses most vascular elements during their functional life, constituents with even the slightest solubility in water would be eluted from the cell lumina and probably also from the cell walls.

By this postulated mechanism, the solubilized constituents would become part of the supply of organic N compounds released by root tissues and carried upward in the transpiration stream to nourish the living tissues of the stem and crown of the tree. These compounds could be reassimilated in the stem by living longitudinal and ray-parenchyma cells adjacent to vascular elements or in the foliage and thus function again in the N economy of the tree. Such an internal recycling mechanism for conservation of N would have survival value for trees comparable in significance with that resulting from the retrieval of N from leaf tissues before abscission (54).

In Fig. 1 we have named the second phase of diminution in N content of wood cells the "elution phase", and shown the changes as a broken rather than a solid line since available evidence in support of this postulated mechanism is less strong than for the other phases. In view of the great rapidity of removal of cytoplasm shown in Fig. 2, the postulated elution phase has been divided into two stages: the first being very rapid and probably involving the bulk of the N in the lumina of vascular elements, and the second being much more gradual and involving the elution of smaller amounts of N probably from the cell walls as well as from the lumina. By the end of the first growing season after cell division, however, the N content of the wood of most tree species has been reduced to 0.06–0.50% by weight (33).

In contrast with the wood fiber cells, the longitudinal and ray-parenchyma cells of the sapwood often remain alive for a number of years. As long as they live, their N content will be maintained at a comparatively high level by the nitrogenous constituents of the cytoplasm. They may function also in storage of N so that their N content could be particularly high in certain seasons of the year. As the sapwood ages, however, more and more of the longitudinal and ray-parenchyma cells die and lose their cytoplasm (8, 28, 54). These changes are coincident with the general decrease in N content across the sapwood (9, 69). For this reason we have used the term "parenchyma death phase" to refer to the general decrease in N content from the first annual increment across the sapwood (Fig. 1). In species in which no true heartwood is formed (e.g., *Populus* sp.), the parenchyma cells die progressively from the first annual increment to those near the pith. In species that contain typical heartwood (e.g., *Quercus* sp.), many parenchyma cells cease to function and lose their cytoplasm as they are transformed from sapwood into heartwood. Although a few living cells have been reported in heartwood (30, 64), the number that remain alive is too small for N in their cytoplasm to influence significantly the N content of heartwood. Removal of cytoplasmic N from parenchyma cells may not be as complete as in the case of wood fiber cells since the former do not participate directly in transpiration.

At the sapwood–heartwood transition zone, the N content reaches a stable level that in most species is between 0.03 and 0.20% N by weight, hence the term "stable phase" covering the bulk of the heartwood in Fig. 1. Tissues in the annual increments near the pith in conifers have been shown to have a slightly higher N content (0.04–0.25% N) than those in the remainder of the heartwood (9, 69). The cells of this core region generally form less secondary cell wall substance than those formed later in the development of the tree. This lesser dilution by cell wall substance may account in part for their higher N content. It also may be due to less complete removal of N from these cells than from those transformed into heartwood later in the development of the tree. It has not been determined whether a similarly higher N content occurs near the pith of angiosperms.

During heartwood formation, various substances of low molecular weight that ordinarily contain little or no N are deposited in the wood. In certain species these substances constitute a significant proportion of the dry weight of the tissue and thus would further reduce the percentage by weight of N in the wood. Since this further dilution of N in wood is significant in only a few tree species, its influence has not been shown in Fig. 1.

Amounts and Gross Distribution of Nitrogen in Wood

The amounts of N shown in Table I are representative of many other values available in the literature for stem wood of various temperate zone species of gymnosperms and angiosperms. A recent preliminary report summarizing unpublished N analyses for a group of 180 species indicates that wood of all but 14 tropical angiosperms contained less than 0.3% N and most were below 0.2% (31). Thus, the amount of N in most wood species is quite small; the C:N ratio for the wood species included in Table I varies from approximately 500–1000 to 1.

The amounts of N reported in various portions of the xylem cylinder of a few tree species is shown in Table II. Although seasonal fluctuations in N content have been reported (36, 54), the season in which the samples were obtained for analysis has not been specified in most of the references cited.

TABLE I

Nitrogen content of stem wood of various gymnosperms and angiosperms. The data shown are for individual samples from a given tree*

Tree species	N content (% by wt.)
Gymnosperms	
Abies concolor	0.045
Abies magnifica	0.227
Juniperus virginiana	0.139
Larix occidentallis	0.180
Libocedrus decurrens	0.097
Picea engelmanni	0.118
Pinus contorta	0.071
Pinus echinata	0.130
Pinus elliottii	0.050
Pinus lambertiana	0.124
Pinus monticola	0.113
Pinus palustris	0.038
Pinus ponderosa	0.052
Pinus strobus	0.087
Pinus taeda	0.068
Pseudotsuga taxifolia	0.051
Sequoia sempervirens	0.067
Taxodium distichum	0.057
Tsuga canadensis	0.106
Angiosperms	
Carya ovata	0.100
Castanea dentata	0.072
Juglans nigra	0.100
Liquidambar styraciflua	0.057
Liriodendron tulipiferae	0.088
Quercus alba	0.104
Quercus rubra	0.099
Quercus stellata	0.096
Quercus velutina	0.070

*Data taken from ref. 5.

TABLE II

Nitrogen content of portions of the stem wood of various tree species

	Nitrogen content (% dry weight)				
Tree species	Cambium	Immature sapwood	Sapwood	Heartwood	Reference
Gymnosperms					
Picea mariana	1.10		0.056	0.059	(7)
Pinus sylvestris	3.25		0.012		(82)
Picea mariana	1.11	0.27	0.047	0.062	(82)
Pinus	3.33		0.130		(6)
Corsican pine, butt			0.108	0.067	(10)
Corsican pine, crown			0.085	0.063	(10)
Pinus sylvestris, butt			0.086	0.079	(10)
Pinus sylvestris, crown			0.052		(10)
Pinus sylvestris		0.115–0.078	0.047–0.039	0.031–0.047	(9)
Angiosperms					
Fraxinus elatior	4.59	0.88	0.22		(82)
Ulmus sativa	4.69	0.81	0.27		(82)
Eucalyptus regnans	2.03	0.52	0.062	0.031	(82)
Fraxinus sp.	4.70	0.89	0.22		(6)
Ulmus sp.	4.80	0.83	0.28		(6)

Most heartwood values in Table II probably are reliable for the tree from which the samples were obtained, since N content does not change appreciably with radial position within the heartwood except at the pith. But values for sapwood should be considered estimates only, since the radial position of the sample analyzed rarely was specified, and in only a few cases were the cambium and first several annual increments removed before analysis (see Fig. 1). Despite these reservations concerning the data of Table II, we conclude in general that a higher percent by weight of N exists: (1) in angiosperms than in gymnosperms (82); (2) within the crown of the tree than below it (74); (3) in sapwood than in heartwood (9, 10); (4) near the cambium than close to the sapwood–heartwood interface (82); and (5) near the pith than in the more recently formed heartwood (9, 69). The N content of rootwood of several conifers was consistently higher than stemwood of the same species (36, 76).

Certain additional inferences about the gross distribution of N in tree stems can be made in the light of factors discussed earlier in relation to the data of Fig. 1. In a tree that forms no true heartwood, the parenchyma death phase presumably would extend all the way to the pith. An older stem with more heartwood than the 40 increments shown in Fig. 1 would show a low stable level of N for a larger number of annual increments. A younger stem with less heartwood would show a narrower band of tissue with a low stable level of N.

The higher N content reported within than below the crown (74) may result from: (1) a real difference in N content with height in tissues of a given stage of maturity; (2) a difference in the proportion of tissues in the parenchyma death phase and stable phase of diminution of N content; and (3) the larger proportion of tissues near the pith, with their higher N content, in samples from within the crown.

Distribution of Nitrogen among and within Wood Cells

Nitrogen occurs in the lumina of wood cells wherever the cytoplasm is functional. Thus, the lumina of immature wood fibers near the cambium and living parenchyma cells of the sapwood would be particularly rich in N. The latter cells may function in storage of nitrogenous constituents and as a consequence would be higher in N content in certain seasons of the year than in others. Since the ray tissues of sapwood contain more living cells than the surrounding wood-fiber cells, ray tissues presumably have a higher N content than vascular tissues.

Nitrogen has been demonstrated to exist as protein in the primary walls of many higher plants. Evidence that it also occurs in primary walls of wood cells is provided by the following observations: (1) amino acids and total N were most abundant in hydrolysates of wood pulp fibers in which primary walls were least degraded during processing (52); (2) a protein called extensin that is believed to function in cell wall extension by providing labile links between cellulose microfibrils has been isolated from callus tissue and alpha-cellulose derived from maple cambium and many other plant tissues (57, 58); and (3) oxidative enzymes, probably peroxidase and (or) phenol oxidase, have been shown to function in polymerization of lignin monomers (27). Peroxidase has been demonstrated in the compound middle lamella of developing wood cells (85). If this enzyme also functions in the synthesis of secondary wall lignin, it probably also is present in the secondary wall, at least during lignification of this layer. It is not known whether cell wall proteins remain within the wall or are removed during the elution phase of diminution in N content (Fig. 1). We believe, however, that at least some of the protein in the primary wall would be resistant to elution by the transpiration stream because of limitations of solubility or accessibility.

Because a larger amount of cell wall substance is laid down in latewood than in earlywood cells and in mature than in juvenile wood cells, the dilution of primary wall N by apposition of secondary wall substance would be greater in the former of these two types of cells than in the latter. Thus, N content would be higher in earlywood than latewood and, within heartwood, higher in juvenile than mature wood cells. In sapwood, the difference in N content of juvenile and mature wood would be obscured by parenchyma cell N. These expectations have been confirmed (9, 69): earlywood of *Pinus sylvestris* L. contained 1.5–2.0 times as much N by weight as latewood although a similar difference was not found consistently in spruce wood; older heartwood (juvenile wood) of *Pinus* and *Picea* spp. contained more N than younger heartwood in the same stem.

Chemical Form of Nitrogen in Wood

With the exception of the alkaloids in a few tree species (65), the chemical form and structure of the nitrogenous constituents in woody plant tissues has not been studied intensively. Nitrogen in wood generally is assumed to be proteinaceous in nature (13, 56, 88). But 50% of the N in heartwood of English oak and one of the *Eucalyptus* sp. and 25% of that in sapwood of the same *Eucalyptus* sp. has been reported to be non-protein (39, 83). In detailed chromatographic analyses of hydrolysates of extractive-free *Pinus* *sylvestris* stem wood, 19 of the common protein amino acids were detected (56). The total amount of the acids decreased from the cambium to the pith but the same acids were found in both heartwood and sapwood. Free amino acids also have been detected chromatrographically in ethanol extracts of heartwood and sapwood of 5 conifers and 19 angiosperms (29). All were normal protein amino acids, although the 12 detected included none of the common aromatic amino acids. The amounts of amino acids in these species also were greater in sapwood than in heartwood.

In addition to proteins and amino acids, all woody tissues that contain living parenchyma cells must contain peptides, nucleic acids, and many other N compounds essential in their metabolism. Indole compounds have been reported in wood and the possibility of a variety of inorganic N compounds cannot be excluded (88). Alkaloids of various types have been reported in 57 of 295 families of gymnosperms and angiosperms (65). Species with unusually high N contents (greater than 0.3% by weight) usually are so because they contain alkaloids that may comprise as much as 5% of the weight of the wood in certain tropical species (88). But with these few exceptions, none of the non-protein and non-amino acid nitrogenous constituents of wood have been characterized or their presence confirmed by analysis.

Nitrogen Metabolism of Wood-Destroying Fungi

In common with all other living organisms, wood-destroying fungi require N in all aspects of their growth and metabolism because it is a constituent of the extracellular and intracellular enzymes, nucleic acids, lipoprotein membranes, chitin, and other constituents of fungus cells. With the exception of certain aspects of the utilization and synthesis of amino acids, the metabolism of N by fungi in general and wood-destroying fungi in particular has been relatively neglected by fungus physiologists despite its importance (16). The following paragraphs summarize existing knowledge of the comparative suitability of various sources of N for growth of wood-destroying fungi, the amounts and distribution of N in fungus tissues, and the influence of N on wood deterioration.

Sources of Nitrogen

Basidiomycetous wood-destroying fungi can utilize ammonia N (43), and a few have been reported to use nitrate N (55, 72) but all have been found to grow most luxuriantly on amino N, among these three forms of the element (43, 44, 49, 70, 71, 72). Urea generally supports less growth than other forms of amino N. Single amino acids differ considerably in the amount and extent of growth they support (43). Only the naturally occurring L forms of the protein amino acids are utilized (45). Combinations of amino acids containing the same total amount of N support greater growth the larger the number of acids in the combination. Complex mixtures of free amino acids and peptides, such as are provided in casein hydrolysate and peptone, support at least as good growth as does malt extract, a standard medium for cultivation of wood-destroying fungi (43). Media containing certain peptides (glycylglycine and glutathione) have been shown to support greater growth than media containing equimolar amounts of their constituent amino acids (72). Thus, proteins may support greater growth than their constituent amino acids because certain peptides are available preformed. Wood-destroying fungi produce proteolytic enzymes (12) and presumably satisfy their N requirements at least in part by hydrolysis of the proteins in wood. To our knowledge, it is not known whether N in the form of nucleic acids, imides, indoles, alkaloids, and other non-amino N compounds that may occur in wood can be utilized as N sources by wood-destroying fungi.

The optimum amount of N for growth of several wood-destroying basidiomycetes in synthetic media was 0.07–0.11% (w/v) when carbon was supplied as 11–12% (w/v) glucose in the medium (44). The C:N ratio in this case was (100–170):1. In wood, however, the C:N ratio is usually (350–500):1, although it can be as high as 1250:1 in spruce heartwood (34).

Nitrogen Content of Fungus Cells

Meaningful generalizations concerning the N content of fungus mycelium and spores are difficult because N content varies so greatly with the species, isolate (38), and tissue of the fungus involved. It varies also with the age, pH, degree of aeration, and composition of the medium on which the fungus is grown, and particularly with the form and concentration of N in the substrate with respect to the amount of carbon available (16, 26). The N content of fruiting bodies of various fungi collected in nature varied from 1.5 to 7% of the dry weight; mycelium of some of the same fungi grown in culture varied from 2 to 6% and averaged more than 4% (35). Mycelium of *Tricholoma fumosum* Fr. contained 5–7% N depending on the substrate (72). When cultivated in typical microbiological media, the N content of vegetative mycelium of most fungi is 3–6% (26). But as the N content of the substrate decreases, the N content of mycelium of most fungi decreases to a minimum seldom less than 2% by weight (35). Under conditions of N starvation, however, N contents as low as 1% have been reported (26). Mycelium of the wood-destroying fungi, *Polyporus versicolor* L. and *Phellinus cryptarum* Karst., growing on wood contained only 0.68% N by weight (15, 24). Thus, at least these two wood-destroying fungi have been shown to contain less N per unit weight of mycelium than other fungi or plants studied to date.

Influence of Nitrogen on Wood Deterioration

Since wood-destroying fungi grow well on wood as sole source of nutrients, they apparently are efficient in obtaining N from the wood and maintaining it within their mycelium without appreciable loss. Evidence that the amounts of N that occur naturally in wood are less than optimal for wood-destroying fungi is provided by observations that addition of small amounts of amino or ammonia N increases the rate and amount of decay in wood blocks (24, 40, 78). But contrary results also have been reported (46, 78). If large amounts of soluble carbohydrates and N are added to wood simultaneously, decay is inhibited until the fungi have consumed the added substrates (66, 79). Naturally occurring N constituents of wood apparently are utilized more efficiently than artificially added N compounds (42). In a comparison of the susceptibility of sapwood from roots and stems of eight coniferous species to decay by isolates of *Fomes annosus* (Fr.) Karst., greater decay susceptibility was associated with larger amounts of N per unit weight in rootwood than in stemwood (76).

Addition of N to an agar medium (63), to soil (90, 91), or to expanded mica (92) or the use of soils high in N (11, 40) resulted in greater weight loss of wood exposed to fungi growing on these media. Soil-inhabiting fungi decay wood rapidly only in the presence of added N. To obtain significant decomposition of hardwood and softwood sawdust, it was necessary to raise its N content to 2% by addition of $NaNO_3$ (1–5). In these studies a straight line correlation ($r = 0.93$) was observed between the amount of N immobilized by incorporation into fungus mycelium and the amount of CO_2 evolved as the result of carbohydrate utilization. Similar correlations have been obtained with other cellulosic materials in soil (37). To obtain substantial deterioration of wood by soft-rot fungi it is necessary to provide additional mineral salts, including N (23, 73, 92).

If the amount of N in wood influences the rate and amount of decay as suggested above, it follows that extraction to remove N should decrease decay. Extraction with water did decrease decay susceptibility in sapwood of certain coniferous species (75, 81).

Mechanisms of Nitrogen Conservation by Wood-Destroying Fungi

Three possible mechanisms may be suggested to account for the ability of wood-destroying fungi to utilize wood as a substrate and to produce the large fruiting structures and enormous numbers of spores observed in nature, despite the meager supply of N in wood: (1) physiological adaptations that result in preferential allocation of available N to metabolically active substances and pathways that are highly efficient in the utilization of wood constituents; (2) reuse of available N by a dynamic and continuous system of autolysis of less active cells and reuse of nitrogenous constituents by more active mycelia without significant loss; and (3) utilization of N from sources outside the wood itself so that the fungi are not dependent upon N in wood as their sole source of supply, for example by fixation of atmospheric N. These postulated mechanisms are not mutually exclusive; any or all of them could be involved. Experimental evidence and logical inferences supporting each mechanism will be discussed in turn.

Physiological Adaptation of Nitrogen Metabolism

Physiological adaptation of organisms in response to selection pressures resulting from nutritional stresses imposed by the environment are well known in plant ecology. The capacity of wood-destroying fungi to adapt their physiological requirements to utilize a less readily degradable substrate, already has been demonstrated in relatively short term laboratory experiments (22). After the ratio of glucose to lignosulfonic acids in the culture had been reduced progressively, *Polyporus versicolor* was able to utilize lignin as sole source of carbon. Physiological adaptations that would result in preferential allocation of available N to metabolic substances and pathways that are highly efficient in the utilization of wood constituents could take several forms: (1) reduction in the amount of chitin, structural proteins, and other metabolically inert nitrogenous substances in cell walls; (2) simplification of metabolic pathways so that a minimum number of different enzyme-proteins (particularly extracellular enzyme-proteins) would be required in the metabolism of wood constituents; and (3) increase in the average turnover number of the enzymes involved in metabolism of wood constituents so that a minimum amount of N would be needed to accomplish a given amount of metabolic work. We have found no evidence in the literature that would directly support these theoretical mechanisms except the comparatively lower N content of *Polyporus versicolor* and *Phellinus cryptarum* grown on wood than that reported for other fungi (15, 24).

Autolysis and Reuse of Nitrogen in Fungus Mycelium

Evidence that wood-destroying fungi may conserve their resources of N by reusing N incorporated in their own mycelium is provided by the common observation that many bore holes formed as the fungi pass from cell to cell within wood are empty or contain only fragments of the hyphae responsible for their formation (17, 25, 41, 47). In nature, bacteria and other fungi could be responsible for the lysis of mycelium. But hyphae also disappear from bore holes in wood blocks sterilized and then inoculated with pure cultures of wood-destroying fungi (17).

In the extensive published literature on autolysis in fungi, wood-destroying organisms rarely have been used as test organisms. Thus, comparatively little is known concerning the specific processes by which wood-destroying fungi may autolyze and reuse their nitrogenous constituents. Most of our present knowledge of autolysis in fungi has been obtained from researches on Fungi Imperfecti such as *Aspergillus niger* and *Penicillium* sp. (16, 51, 67, 68, 80). In the species studied, autolysis usually is initiated in response to exhaustion in the substrate of some essential nutrient, particularly carbon or nitrogen. In liquid cultures, autolysis is associated with a decrease in dry weight of mycelium and release into the medium of various N compounds including ammonia, amino acids, peptides, proteins including certain enzymes, and other soluble materials. In the presence of suitable carbohydrates or other utilizable sources of carbon, certain of these products have been shown to be reassimilated and used again to support continued growth of the mycelium (51). When N in the medium is exhausted, further growth of *Scopulariopsis brevicaulis* can take place by mobilization and reutilization of N in older hyphae (68).

From the above we conclude that the meager supply of N available in wood could provide the stimulus for autolysis of wood-destroying fungi and that autolysis and reuse could occur by either internal or external mechanisms, i.e. (1) internal translocation of cytoplasmic constituents or their autolytic products from older cells to younger, more active cells; or (2) extracellular release of enzyme systems that depolymerize the cell walls and cytoplasmic constituents of less active cells to soluble products that can be assimilated and reused by other cells.

Utilization of Nitrogen Sources outside the Wood Itself

In many conditions of exposure, the N requirements of wood-destroying fungi can be satisfied by sources outside the wood itself. Under conditions of soil contact, for example, the fungi could assimilate N directly from the soil.

Also in living trees, decay fungi may obtain N from the transpiration stream of the tree itself. Such exterior sources may contribute a substantial proportion of the total N required by the fungi. But under conditions of aboveground exposure such as in buildings, standing dead trees, fallen logs that are not in contact with the ground, and in laboratory experiments in which wood is exposed without contact with accessory nutrient substances, the only source of N outside the wood itself would be the atmosphere. Fixation of atmospheric N often has been suggested, but to our knowledge never proved to occur in fungi (16). Recent measurements of the amount of N in wood in progressive stages of decay by pure cultures of *Merulius domesticus* failed to provide evidence for fixation of N by this wood-destroying fungus (50). But the demand of wood-destroying fungi for N during decay undoubtedly is much greater when sporophore formation and sporulation are involved than when not involved. Nitrogen liberated in spores is lost permanently from the fungus–wood ecosystem; that in sporophores certainly is not retrieved *in toto*, since sporophores contain some N, but the possibility exists that some N may be retrieved from fruiting structures. Thus, fixation of atmospheric N is more likely to occur during sporophore formation and sporulation than when the fungi are involved merely in the vegetative phases of their life cycle. To our knowledge, no tests of possible fixation by wood-destroying fungi have been made by the definitive methods involving ^{15}N (14).

Conclusion

In this paper we have attempted to synthesize from existing knowledge of wood and cambial chemistry as well as that of tree and fungus physiology, a realistic understanding of N in wood and its probable role in wood deterioration. We have presented a number of working hypotheses that are being used as a basis for experimental work currently underway to expand our knowledge of the amounts, chemical forms, and distribution of N in wood and in wood-destroying fungi and to determine the mechanisms wood-destroying fungi have evolved to degrade wood constituents and sporulate on wood despite its paucity of N. The parallel between the hypothesized mechanisms for internal recycling of N in trees and autolysis and reuse in wood-destroying fungi provide an intriguing possibility of comparative and concomitant evolution in these two types of organisms.

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